Neuroprotective effects of metalosalen complexes against oxidative stress

Lara Rouco, Marcelino Maneiro*

Neurodegenerative diseases and oxidative stress: During the metabolic processes, O_2 can accept unpaired electrons to form superoxide radical species ($O_2^{\bullet -}$), which are able to generate hydrogen peroxide (H_2O_2) with a fast dismutation, therefore increasing the hydroxyl radical ($HO^{\bullet}$) levels. These species and other radicals ($^{\bullet}OOH$, $ROO^{\bullet}$, $RO^{\bullet}$, $CO_3^{\bullet -}$), which can be produced through a sequence of reactions, constitute the designated reactive species of oxygen (ROS). Oxidative stress is caused by a disequilibrium between the ROS produced and the antioxidant defence against them (catalase enzymes, superoxide dismutase (SOD) and glutathione peroxidases, in addition to other non-enzymatic antioxidants, such as α-tocopherol, ascorbic acid and carotenes). The consequences of oxidative stress are the increase in the formation of oxidized cellular macromolecules, the activation of phagocytes, the release of cytokines or the activation of oncogenes. These processes lead to different pathologies in humans, such as carcinogenesis, inflammatory illnesses, diabetes type II, cellular senescence and different neurodegenerative diseases (Zhao et al., 2019). The brain is prone to oxidative stress since neurons consume large amounts of oxygen (20% of oxygen uptake when brain accounts for only 2% of body weight) due to the high rate of energy consumption (4×10^{12} ATP/min) to maintain neuronal intracellular ion homeostasis (Miller et al., 2017). Furthermore, neural mitochondria generate large amounts of hydrogen peroxide compared to skeletal muscle mitochondria. Additionally, neuronal membranes have high concentrations of polyunsaturated fatty acids like arachidonic acid, docosahexaenoic acid or eicosapentaenoic acid; all of them with unsaturated double bonds which are susceptible to oxidation, giving rise to lipid hydroperoxides as the primary oxidation products. Thus, unsaturated lipid peroxidation also contributes to the progression of disbalanced redox homeostasis, generating reactive oxygen species. The effects of oxidative stress are implicated in the progression of several neurodegenerative diseases: chronic diseases like Parkinson's or Alzheimer's diseases, acute injury of the brain like brain trauma or cerebral ischemia, and psychiatric disorders like depression, schizophrenia or autism (Chen et al., 2012).

Antioxidant enzymes: The first line of the antioxidant defence of the organisms is fundamentally based in the SOD and catalase (CAT) enzymes. The global role of the cellular antioxidant defences is the reduction of ROS to water. The SOD catalyses the formation of O_2 and H_2O_2 from two $O_2^{\bullet -}$ radicals. Different types of SOD can be distinguished depending on the metallic cofactor: FeSOD, CuZnSOD, MnSOD and NiSOD. The metal ion is coordinated by donor atoms (mainly nitrogen atoms but also oxygen and sulphur atoms) from amino acid residues. The SOD2 mitochondrial enzyme has manganese in its reactive centre, bound to three histidine ligands, one aspartate and water or hydroxide as the fifth ligand in a trigonal bipyramidal geometry (**Figure 1A**), and carries out $O_2^{\bullet -}$ disproportionation through a redox process involving one-electron oxidation and reduction of the metal ion between Mn(II)/Mn(III) levels. Catalases catalyse the formation of O_2 and H_2O from two H_2O_2 molecules. Common catalase is a tetramer with four iron-containing heme groups, which are able to decompose hydrogen peroxide. The enzyme cycles between Fe(III) and $Fe(IV)^{\bullet +}$ during turnover. Alternatively, non-heme catalases have been found in microbial life. That is the case of the manganese catalases that contain a binuclear manganese catalytic active site (**Figure 1B**) with a two-electron oxidation-reduction cycle during turnover. Despite of the efficiency of these systems (SOD/CAT) against ROS, oxidative stress arises as result of an imbalance in the pro-oxidant/antioxidant homeostasis leading to the generation of toxic levels of ROS. Antioxidant therapy has attracted intensive attention for the treatment of ROS-related neurodegenerative diseases. Administration of exogenous native antioxidant enzymes has not been successful for therapeutic treatment of oxidative stress mainly because of the short half-life of the enzymes and their high molecular weight that prevent them to enter the cells. Antigenicity and high-manufacturing costs are also negative aspects for use the native enzymes. To overcome these limitations, pharmacological research has pointed at the development of low molecular weight SOD/CAT mimics.

Therapeutic catalytic antioxidants: The strategy of obtaining synthetic models that mimic the activity of the antioxidant enzymes, using metallic centres with redox activity, has been developing since the 1990s. An ideal mimetic should be stable and non-toxic. Among the available metallic ions, the manganese seems to be the most adaptable transition metal, as an artificial mimic of the SOD, for pharmaceutical applications because of its relative low toxicity. Three major classes of these compounds are manganese porphyrins, manganese corroles and manganosalen complexes. Manganosalen complexes offer broad structure-function correlation opportunities that have efficacy in several oxidative stress models of human disease. They are cell-permeable and show potent SOD and CAT mimetic activity. Unlike enzymes, manganosalen complexes have higher stability, reduced cost, and they exhibit less environmental sensitivity. *Salen* is the acronym for N,N-bis(salicylidene)ethylendiamine and its derivatives (**Figure 1C**). Although strictly *salen* is the acronym for the ligand formed after condensation of two parts of salicylaldehyde and ethylenediamine, other diamine precursors (like propylenediamine, phenylenediamine) are usually included in this family. These inexpensive ligands resemble those in enzymes, and they coordinate manganese ions through oxygen and nitrogen atoms, which contrasts with corroles and porphyrins where the manganese is coordinated to nitrogen atoms only. After coordination, manganosalen complexes are stable, with different coordination states, which are thought to be important in the scavenging of ROS. Valence states, coordination numbers and geometries depend on the substituents on the phenyl rings of the salen moiety. The SOD activity of the manganosalen complexes proceed via the Mn(II)/Mn(III) redox couple and their activities range around 10^6 M^{-1} s^{-1}. The catalase rates for manganosalen complexes are similar to those reported for metalloporphyrins, but obtained using "test tube" H_2O_2 disproportionation, which might not be relevant in more-complex biological systems. The kinetic mechanism for H_2O_2 decomposition involves the formation of an oxomanganese(V)-*salen* intermediate. Moreover, in humans, the level of hydrogen peroxide in cells is maintained through a cooperative action of catalases and glutathione peroxidases, and some manganosalen complexes have also shown high peroxidase activities. In *in vivo* models, it has been proven the beneficial effect of these enzyme mimetics in the protection against radiation injuries or inflammation, and their protective effects on different organs, which include the lung, liver, and kidney (Doctrow et al., 2016). Significant advances have been achieved in establishing the factors involved in the obtention of efficient manganosalen models against oxidative stress (Signorella et al., 2018). Among these factors: the redox properties of the complexes can be modulated by the structure of the *salen* ligands, auxiliary ligands alters the geometry of the compounds, supramolecular interactions afford tools for controlling selectivity and blood-brain barrier permeability, other factors like *in vivo* stability and cytotoxicity may be tuned by an appropriate design of the artificial systems.

Manganosalen SOD/CAT mimetics as therapeutic agents for neurodegenerative diseases: The neuroprotective properties of manganosalen complexes were first reported in the 1990s with different trials with the EUK series, developed by Eukarion (Doctrow et al., 2016). EUK-8 prevented damage produced by acidosis and anoxia in hippocampal slices, inhibited ROS formation and blocked Aβ-induced neurotoxicity in organotypic hippocampal culture, and ameliorated inflammatory autoimmune encephalomyelitis in mice. EUK-134 has shown to be effective in attenuate the oxidative stress and neuropathology in the rat limbic system with seizure-induced hippocampal injury. In the first decade of the 21st century, other manganosalen complexes demonstrated beneficial effects against different neurological pathologies in various *in vitro* and *in vivo* models, like a mouse model suffering from amyotrophic lateral sclerosis, a mouse model with paraquat-mediated SNpe dopaminergic neuronal cell death (with implications for Parkinson disease), a sod2nullizygous mice model with spongiform neurodegenerative disorder, a rodent stroke model with permanent regional cerebral ischemia, or a mouse model of human prion disease (Doctrow et al., 2016). Other recent studies have expanded the neuroprotective effects in age-related cognitive impairment in mice (Clausen et al., 2010), in radiation-induced cognitive injury in sham-irradiated animal models (Raber et al., 2017), in oxidative DNA damage and senescence phenotypes in cochlear cells (Benkafadar et al., 2019),

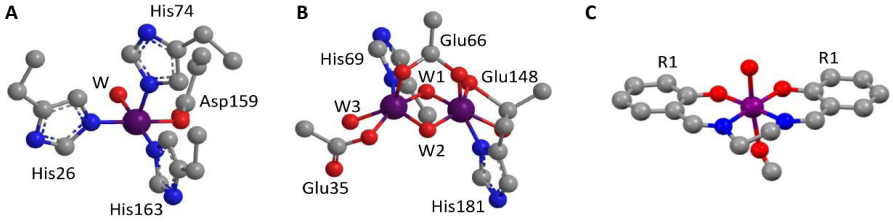

Figure 1 | **View of the coordination environment for manganese ions in SOD/CAT enzymes and manganosalen complexes.**
(A) Core of the active site of human mitochondrial SOD2; (B) Core of the active site in *Lactobacillus plantarum* catalase; (C) manganosalen model complex. CAT: Catalase; SOD: superoside dismutase; W: water.

and against oxidative stress in human SH-SY5Y neuroblastoma cells (Rouco et al., 2020). Many of these studies show a response of the beneficial effects of the manganosalen complexes against different damages and relate it to their SOD/CAT activity, though the mechanism of action is still unclear. In fact, the neuroprotective effects of these complexes may be also associated to other roles found for these compounds, like their reactivity towards lipid peroxides or their capacity to control nitrosative stress since they can break down peroxynitrite and nitric oxide to more benign species (Batinic-Haberle et al., 2014). However, a systematic revision of the antioxidant activity of a high number of manganosalen complexes in different trials has been very useful to establish some guidelines, for example, the fact that low molecular weight monomeric complexes can enter the cells. Moreover, the antioxidant activity for this type of complexes increases when the organic ligand possess: i) a short carbon chain between imine groups, since this feature leads to square-pyramidal or tetragonally elongated octahedral geometries, that have either a vacancy or a labile ligand in the coordination sphere of the metal ion; subsequently a substrate molecule, like hydrogen peroxide, may be accommodated in this coordination position; ii) alkoxy substituents in the phenyl rings, particularly 3-methoxy groups (position R1 in **Figure 1C**), since the electron-donor character of these substituents lowers the redox potentials for the manganosalen complexes, and facilitates to achieve higher oxidation states for the manganese ion during enzymatic activity. Methoxy groups can also participate in establishing new supramolecular interactions through hydrogen bonding that not only induce self-organization of the complexes to afford dimeric entities, but also play an essential role in recognition in biological processes.

Perspective: The World Health Organization estimates that, by 2040, neurodegenerative diseases will be the main cause of death in industrialized countries ahead of the cancers. The accelerated ageing of the population will be accompanied with a rise in the number of patients affected by a neurodegenerative disease. Extensive studies pointed out the role of oxidative stress in several type of neurodegenerations, and this type of disorder accumulates with aging. In this context, it is necessary to redouble efforts towards the search of antioxidant enzyme mimics. Manganosalen complexes possess some important features that make them suitable for developing drugs for this purpose. Although significant antioxidative activities have been achieved in oxidative stress models *in vitro* and *in vivo*, their practical application in humans remains highly challenging. Future investigations should focus on this regard; clinical trials with humans are crucial. For instance, different antioxidants supplements like coenzyme Q10, creatine, α-tocopherol, β-carotene, etc., showed highly encouraging results *in vitro* and *in vivo* animal models but most clinical trials in humans fail to reproduce positive results (Grodstein el al, 2013; Oertel et al, 2016). Conversely catalytic antioxidants may regulate ROS by mimicking the mechanism of enzymatic action of native SOD/CAT. Clinical trials are needed to assess the efficacy of this approach. Manganosalen complexes offer broad opportunities to manipulate their structure and coordination chemistry investigations. In this sense, some issues actually need more research, to quote only three of them: i) it is important to look into how bioactivity is correlated with the intracellular concentration and distribution of the exogenous manganosalen complexes, new developments in imaging based in MRI technology are available to address this issue; ii) the role of supramolecular interactions in the bioactivity of these compounds is not yet fully understood, for instance, supramolecular mechanisms may allow aggregation of the complexes into dimers once the monomers cross the cell membrane, consequently, dimerization equilibria caused by supramolecular interactions have to be taken into account; iii) much effort has been directed towards obtaining mimics that have both SOD and CAT activity, but new recent findings open up other interesting antioxidant pathways for manganosalen complexes, like a cascade mechanism driven by a complex with only SOD activity, which, once it acts as radical scavenger gives rise to another species with CAT activity (Liberato et al., 2018); thus, redox side effects within the cell can be avoided since catalase function is activated only after radical reaction.

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